REMARKS

Claims 24-28, 32-35, 37-44, 55-57 and 59-72 have been canceled, and new Claims 81-103 have been added to point out with more particularity and clarity the subject matter regarded by the Applicant as his invention. In adding new Claims 81-103 and in canceling Claims 24-28, 32-35, 37-44, 55-57 and 59-72 to focus with more particularity and clarity on one aspect of the invention as originally filed, Applicant respectfully reserves the right to file subsequent application(s) to protect the invention commensurate with the scope as originally filed.

New claims 81-103 basically correspond to claims 24-28, 33-35, 37, 72, 40-44, 55-57, 59, 60, 63, 32 and 61, respectively. Support for modifications from the respective corresponding claims is detailed below.

New Claim 81, the only presently pending independent claim, is based upon canceled Claim 24 but, instead of being directed to "[a] method of detecting a disease or disease susceptibility trait," is directed to "[a] method of screening for hereditary colorectal cancer or a predisposition to hereditary colorectal cancer. . . ." Support for "[a] method of screening for hereditary colorectal cancer or a predisposition to hereditary colorectal cancer" can be found throughout the instant specification but at least at page 1, line 17 to page 5, line 15, particularly at page 3, lines 3-11, which reads:

> Representative immunoassays are disclosed which are used to screen primarily for certain types of hereditary colorectal cancer (CRC) or a predisposition to hereditary CRC. Such representative immunoassay methods to screen for hereditary CRC or a predisposition thereto are based on the detection of cellular full-length protein level changes that are due to either (1) mutations of the adenomatous polyposis gene (APC), a mutation that is associated with familial adenomatous polyposis (FAP), or to (2) mutations of mismatch repair (MMR) genes, particularly, MLH1, MSH2,

> PMS1, PMS2, and MSH6, mutations that are associated with hereditary non-polyposis colon cancer (HNPCC).

Further support can be found in the specification at least at page 6, line 3 to page 7, line 5; at page 8, lines 3-22; at page 14, lines 19-29; at page 18, line 10 to page 30, line 14, particularly at page 18, line 10 to page 19, line 24; at page 38, line 30 to page 39, line 15; and at page 45, line 21 to page 59, line 20 (Examples 1-4).

The language in canceled claim 24 which describes the type of "disease" or "disease susceptibility trait" detected as being associated with germline mutations that cause "an about 50% decrease in the level of wild-type protein expressed by one of two or more subject genes in affected individuals . . ." is not present in new claim 81, as such language is not necessary to describe "hereditary colorectal cancer or a predisposition to hereditary colorectal cancer. . . ." Subclaim (d) of canceled claim 24, that is,

> . . . (d) immunologically quantitating the amount of wild-type protein expressed by each of the subject genes in said protein extract of said normal cells; . . .

is replaced by the revised subclaim (d) of new Claim 81:

> . . . d) immunologically quantitating the levels of two or more wild-type subject proteins in said protein extract of said normal cells, wherein said subject proteins are selected from the group consisting of MLH1, MSH2, MSH6, PMS1, PMS2, and APC proteins; . . . .

To point out with greater particularity and clarity that the methods of the invention are to protein-based screening assays to detect colorectal cancer or colorectal cancer susceptibility and not to DNA-based, molecular genetic sequencing assays, new Claims 81-103 refer throughout to quantitating "subject proteins" rather than to quantitating "proteins expressed from subject genes." Support for those amendments

can be found throughout the specification but particularly at least at page 2, line 24 to page 3, line 2, which reads:

> The assays of this invention are based on the assumption that gene expression directly relates to gene dosage, that is, the presence of two wild-type alleles will result in the expression of twice the amount of full-length wild-type protein than would occur if only one wild-type allele were present. In accordance with this invention, immunoassays are used to measure a reduction from normal in the amount of full-length protein expressed by a subject gene. In contrast, mutations in genes, such as mismatch repair (MMR) genes, are classically detected by DNA sequence analysis and/or in vitro translation-type assays [Giardiello, F.M., "Genetic Testing in Hereditary Colon Cancer," JAMA, 278: 1278-1281 (1997)], tests which are costly, time consuming and only offered in select academic and commercial reference labs.

Further support for new claims 81-103 can be found at least at page 3, lines 24-27, which reads: "Thus, the immunoassays of this invention may become useful as a complementary 'pre-test'. This invention may also support molecular genetic tests in the diagnosis of affected members of known kindreds in which the mutation has already been identified." [Emphasis added.]

Applicant respectfully concludes that no new matter has been entered by the cancellation of Claims 24-28, 32-35, 37-44, 55-57 and 59-72 and by the addition of new Claims 81-103.

35 USC 112, 1st Paragraph Rejection

Claims 24-28, 32-35, 37-44, 55-57 and 59-72 stand rejected under 35 USC 112, first paragraph for reasons of record, and because "[t]he claims, as currently amended are not fully described by the specification, and therefore, introduce new

matter in to the specification. . . ." [Office Action of March 18, 2005, page 3, Section 4.] Applicant respectfully submits that the amendments to the claims overcome the 35 USC 112, 1st paragraph rejection.

The Office Action of March 18, 2005 [hereinafter cited as "Office Action"] at page 3 bases the subject 112 first paragraph rejection on a claim phrase, not in new claims 81-103 but only in now canceled independent claim 24. That now absent phrase is "wherein said disease or said disease susceptibility trait has been associated with a germline mutation that causes an about 50% decrease in the level of wild-type protein." Applicant respectfully points out that that phrase has been deleted from new claim 81, the only presently pending independent claim. The Examiner in the Advisory Action of August 3, 2005 stated that an amendment deleting that phrase would overcome the subject 112, first paragraph rejection, and Applicants respectfully agree that the deletion of that phrase from the pending claims overcomes the subject rejection.[1]

The Office Action states on page 3, that

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1. The Advisory Action mailed from the PTO on August 3, 2005 states under "Continuation of 5" that Applicant's Amendment After Final mailed to the PTO on May 18, 2005

> has overcome the following rejections(s): Rejection of claims 24-28, 32-35, 37-44, 55-57 and 59-72 under **35 U.S.C. 112**, first as failing to comply with the written description requirement **would be overcome by this amendment, if entered, because the amendment removes that phrase "wherein said disease or said disease susceptibility trait has been associated with a germline mutation. . .".**

[Emphasis added.] Since the instant amendment similarly removes from the pending claims the phrase "wherein said disease or said disease susceptibility trait has been associated with a germline mutation . . . ," Applicant respectfully

> [t]he amendment introduces new matter because the phrase "wherein said disease or said disease susceptibility trait has been associated with a germline mutation that causes an about 50% decrease in the level of wild-type protein" has no support in the specification as originally filed. The phrase "has been associated" implies a time frame for the "association". The specification fails to discuss this concept. Is the association as of the current amendment or is the association as of the effective file date of the application?
>
> . . . Applicant points to a passage on page 2, lines 24-27, where the specification teaches that assays of the invention are based on the assumption that gene expression directly relates to gene dosage. However, ["] wherein said disease or said disease susceptibility trait **has been associated with** a germline mutation that causes an about 50% decrease in the level of wild-type protein" is broader in scope because an "association" [can] be one that exist[s] because of theoretical assumption, as alluded to in the specification, or because of an empirical finding. Therefore, the amendment introduces new matter into the specification because the phrase "has been associated" is different in scope from the concept presented in the specification of a theoretical association between a heterozygous mutation that may produce an about 50% decrease in a level of protein.

[Emphasis added.] Applicant respectfully points out that the subject 112, first paragraph rejection does not apply to new claims 81-103 because the claim scope issue presented in the Office Action concerning "theoretical" verses "empirical" association is rendered moot by the absence of the phrase containing the "has been associated with" language from the only independent new claim 81.

Applicant further respectfully points out, as detailed above in the Remarks section, that the instantly claimed methods of screening for "hereditary colorectal cancer or a predisposition to heredity colorectal cancer" are very well supported in the instant

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agrees with the Examiner's reasoning that the subject 112 , first paragraph amendment is overcome.

specification. For example, the Examples describe methods to screen for hereditary colorectal cancer or predisposition thereto using immunoassays to detect reduction in the levels of either wild-type APC protein (Example 1) or wild-type mismatch repair proteins (Examples 2-4).

Applicant respectfully concludes that the claims as amended comply with the written description requirement of 35 USC 112, first paragraph. Applicant respectfully requests that the Examiner reconsider the subject 112, first paragraph rejection in view of the amendments to the claims and the above remarks, and withdraw the subject rejection.

35 USC 103(a) Rejection

Claims 24-28, 32-35, 43, 44, 55-57 and 61 stand "rejected under 35 USC 103(a) as being unpatentable over Pece (Pece, N. et al. J. Clin. Invest. 100(10):2568-2579, 1997, November; cited in IDS) in view of Nozawa (U.S. Patent 5,328,826; issued July 12, 1994; filed March 23, 1992)." [Office Action of March 18, 2005, page 4, Section 5]. Applicant respectfully traverses the instant rejection pointing out that Nozawa is nonanalogous art, and that Pece is inapposite.

Further, Applicant respectfully points out that the claims have been amended to focus on one aspect of the invention, that is, to methods of screening for "hereditary colorectal cancer or predisposition to hereditary colorectal cancer," wherein the subject proteins are MLH1, MSH2, MSH6, PMS1, PMS2, and APC proteins, all of which are known to be related to colorectal cancer. The pending claims are clearly far removed from the concept of "housekeeping proteins," since each of the subject

proteins is colorectal cancer-related and could be the protein whose level, if found to be reflected in the determined ratio(s) as being at about 50% of normal, indicates the presence of hereditary colorectal cancer or susceptibility thereto.

Nozawa

The Office Action of March 18, 2005 [hereinafter cited as the "Office Action"] states:

> Nozawa is cited for the purpose **of demonstrating that methods for quantitating protein are known in the art and the use of a ratio of level of a protein of interest to a level of a housekeeping protein is known.**
>
> **The claimed inventions include within their scope methods where one of the two subject genes is a housekeeping gene**, and therefore, read on methods where one of the protein[s] detected is detected for the purpose of ensuring that differences observed in protein levels between different samples is due to an actual difference in protein levels per cell and not due to an experimental artifact such as different loading between samples. This is one of the problems addressed in Nozawa (see col, line 44-48 and col. 3, lines 18-52). Therefore, Nozawa is analogous art.

[Emphasis added.] Applicant respectfully ponts out that the scope of new claims 81-103 does not include that wherein a subject protein is "a housekeeping protein" and that Nozawa is not applicable to the subject claim set.

Further, Applicant respectfully submits that Nozawa is not analogous art. Nozawa describes the quantification of a cancer antigen isolated from cancer cells, not normal antigen isolated from normal cells as in the claimed invention. The problems with quantitating antigens for the detection of disease, that Nozawa addresses relate to false positives resulting from subjective interpretation of immunohistochemical staining

of cells, nonspecific binding of the detecting anti-cancer antigen antibody to normal cells (giving rise to false positives), and the difficulty in collecting samples of a constant volume (and therefore, of a constant cell number) for the cells in question, endometrial cells or from tissue from the uterine cavity [Nozawa et al., col. 2, lines 44-51]. None of those problems relate to the present invention.

According to the methods of the present invention, all normal tissues express the antigen in question, that is, the reverse situation from Nozawa. For the preferred type of cells used in the present invention, peripheral blood lymphocytes, a constant volume of cell sample is easily obtainable. The ratio of Nozawa is used to determine the "signal-to-noise" ratio of the specific antigen-antibody staining relative to background staining, in order to eliminate findings of false positives resulting from background staining (due to nonspecificity of antibody, interfering components in the specimen, etc.). Therefore, the cutoff value of Nozawa et al. refers to ratios above a certain "apparent cancer antigen"-to-housekeeping antigen ratio, representing background noise. Claim 5 of Nozawa reads: "wherein a positive result is indicated by a ratio larger than a predetermined value."

The present invention, however, does not use the ratio as a lower limit cutoff, but a defining value of about 50%, indicating that the ratios of Nozawa et al. and the present invention refer to two different phenomena. In a normal cell sample, in the ratio of **y/x** of Nozawa, the signal intensity **y** would represent nonspecific anti-cancer antigen antibody binding to normal cell components (**Ab1**), relative to signal intensity **x** representing primarily specific antibody binding to a housekeeping gene product (**Ab2**). Therefore, in a normal cell, the Nozawa ratio might be designated:

Nonspecific **Ab1** binding

Specific **Ab2** binding + nonspecific **Ab2** binding

According to the present invention, in a normal cell sample, both components of the ratio represent primarily specific antibody binding:

Specific **Ab1** binding + nonspecific **Ab1** binding

Specific **Ab2** binding + nonspecific **Ab2** binding

Here, the ratio is used to determine the normal levels of wild-type protein expression of the subject gene in normal cells.

To summarize, the use of a ratio of antigens in Nozawa et al. is different from that of the present invention, and is nonanalogous art. Further, Nozawa is particularly nonapplicable to the claims in that Nozawa concerns the use of "a housekeeping protein" which concept is not relevant to the instant claims that only concern subject proteins MLH1, MSH2, MSH6, PMS1, PMS2 and APC proteins, none of which are "housekeeping proteins," and all of which are known to be related to hereditary colorectal cancer. Applicant respectfully submits that there is nothing in Nozawa that adds to the disclosure of Pece to render the present invention obvious.

Pece

Applicant respectfully submits that the ratios cited in Pece are not the same ratios as those of the pending claims, first because Pece does not refer to a method of screening for hereditary colorectal cancer or to any of the subject proteins of the pending claims. Secondly, the three proteins assayed in Pece are not analogous to the subject proteins of the instant claims because Pece has only a single subject protein

(endoglin) associated with hereditary disease, whereas the instant invention requires at least two subject proteins associated with hereditary disease: "immunologically quantitating the levels of two or more wild-type subject proteins . . . , wherein said subject proteins are selected from the group consisting of MLH1, MSH2, MSH6, PMS1, PMS2, and APC. . . ." [new Claim 81].

The reference proteins of Pece, $\alpha 5\beta 1$ integrin and CD31, are not associated with any hereditary disease, and certainly not to the germline disease hereditary hemorrhagic telangiectasia type 1 (HHT1), the hereditary disease which is associated with the subject endoglin protein, and is the subject of Pece. A 50% reduction in the levels of either $\alpha 5\beta 1$ integrin or CD31 relative to endoglin would not be an indication of hereditary hemorrhagic telangiectasia type 1 (HHT1). In Pece and Nozawa, a reference protein is detected because it is expected to be unchanged [Pece et al., page 2573, 1st col.], that is, a "substance present in human cells in a substantially constant amount . . ." as expressed in the abstract of Nozawa.

In the pending claims, each and every subject protein is detected because it is suspected of being reduced in level. Pece does not refer to the reduction of the expression of any second or more proteins as a possible cause of HHT1. Further, Pece does not suggest determining a ratio of endoglin to ALK-1 [activin receptor-like kinase 1 related to HHT2 (Pece at page 2568, col. 2)] in families with any type of hereditary hemorrhagic telangiectasia.

In Item 7 of the Advisory Action, the Examiner mistakenly contends that the Applicant previously argued that not all of Pece's subject proteins are "disease associated," and found the Applicant's argument not persuasive because

> . . . the phrase "associated with a disease" may be broadly interpreted. In the case of Pece, a ratio is made of endoglin expression to alpha-v/beta-5 integrin expression. The argument could be made that both of these genes are "disease associated", because a mutation in endoglin is known to occur in a "vascular disorder", and alpha-v/beta-5 integrin is also associated with vascular disorders, such as angiogenesis.

[Advisory Action, Continuation of Item 7.] However, Applicant respectfully points out that that statement from the Advisory Action is in error because it does not distinguish between simply "disease-associated" genes versus hereditary (germline) disease associated genes, the latter being the subject of the claims prior to the instant amendment. Even more strongly, does that Advisory Action not pertain to the instant claims which are directed to one particular hereditary disease – hereditary colorectal cancer and a predisposition to hereditary colorectal cancer. The reference proteins of Pece, $\alpha 5\beta 1$ integrin and CD31, are not related to any hereditary disease, and certainly not to hereditary colorectal cancer or a predisposition to hereditary colorectal cancer.

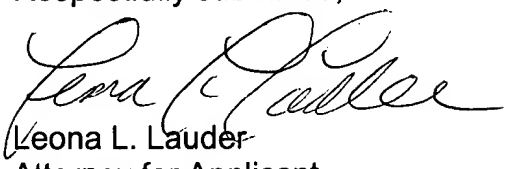
Applicant respectfully concludes that neither Pece nor Nozawa alone nor in combination renders the instantly claimed invention obvious. Applicant respectfully requests that the Examiner reconsider the subject 103(a) rejection in view of the amendments to the claims and the above remarks, and withdraw this rejection.

CONCLUSION

Applicant respectfully concludes that the claims as amended are in condition for allowance, and earnestly requests that the claims be promptly allowed. If for any reason the Examiner feels that a telephone conference could be helpful, the

Examiner is invited to telephone the undersigned Attorney for Applicant at (415) 981-2034.

Respectfully submitted,



Leona L. Lauder
Attorney for Applicant
Registration No. 30,863

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